PCT

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification [6] : **A61K 9/14**	**A1**	(11) International Publication Number: **WO 97/14407** (43) International Publication Date: 24 April 1997 (24.04.97)

(21) International Application Number: PCT/US96/16841

(22) International Filing Date: 17 October 1996 (17.10.96)

(30) Priority Data:
     60/005,340   17 October 1995 (17.10.95)   US

(71) Applicants (*for all designated States except US*): RESEARCH TRIANGLE PHARMACEUTICALS [US/US]; Suite 201, 4364 Alston Avenue, Durham, NC 27713 (US). BOARD OF REGENTS, THE UNIVERSITY OF TEXAS SYSTEM [US/US]; 201 West 7th Street, Austin, TX 78701 (US).

(72) Inventors; and
(75) Inventors/Applicants (*for US only*): HENRIKSEN, Inge, B. [NO/US]; Research Triangle Pharmaceuticals, Suite 201, 4364 Alston Avenue, Durham, NC 27713 (US). MISHRA, Awadesh, K. [IN/US]; Research Triangle Pharmaceuticals, Suite 201, 4364 Alston Avenue, Durham, NC 27713 (US). PACE, Gary, W. [US/US]; Research Triangle Pharmaceuticals, Suite 201, 4364 Alston Avenue, Durham, NC 27713 (US). JOHNSTON, Keith, P. [US/US]; University of Texas, Dept. Chemical Engineering, 26th and Speedway, Austin, TX 78712-1062 (US). MAWSON, Simon [US/US]; University of Texas, Dept. Chemical Engineering, 26th and Speedway, Austin, TX 78712-1062 (US).

(74) Agent: CRAWFORD, Arthur, R.; Nixon & Vanderhye P.C., 8th floor, 1100 North Glebe Road, Arlington, VA 22201-4714 (US).

(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

---

(54) Title: INSOLUBLE DRUG DELIVERY

(57) Abstract

Particles of water insoluble biologically active compounds, particularly water-insoluble drugs, with an average size of 100 nm to about 300 nm, are prepared by dissolving the compound in a solution then spraying the solution into compressed gaz, liquid or supercritical fluid in the presence of appropriate surface modifiers.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AM	Armenia	GB	United Kingdom	MW	Malawi
AT	Austria	GE	Georgia	MX	Mexico
AU	Australia	GN	Guinea	NE	Niger
BB	Barbados	GR	Greece	NL	Netherlands
BE	Belgium	HU	Hungary	NO	Norway
BF	Burkina Faso	IE	Ireland	NZ	New Zealand
BG	Bulgaria	IT	Italy	PL	Poland
BJ	Benin	JP	Japan	PT	Portugal
BR	Brazil	KE	Kenya	RO	Romania
BY	Belarus	KG	Kyrgystan	RU	Russian Federation
CA	Canada	KP	Democratic People's Republic	SD	Sudan
CF	Central African Republic		of Korea	SE	Sweden
CG	Congo	KR	Republic of Korea	SG	Singapore
CH	Switzerland	KZ	Kazakhstan	SI	Slovenia
CI	Côte d'Ivoire	LI	Liechtenstein	SK	Slovakia
CM	Cameroon	LK	Sri Lanka	SN	Senegal
CN	China	LR	Liberia	SZ	Swaziland
CS	Czechoslovakia	LT	Lithuania	TD	Chad
CZ	Czech Republic	LU	Luxembourg	TG	Togo
DE	Germany	LV	Latvia	TJ	Tajikistan
DK	Denmark	MC	Monaco	TT	Trinidad and Tobago
EE	Estonia	MD	Republic of Moldova	UA	Ukraine
ES	Spain	MG	Madagascar	UG	Uganda
FI	Finland	ML	Mali	US	United States of America
FR	France	MN	Mongolia	UZ	Uzbekistan
GA	Gabon	MR	Mauritania	VN	Viet Nam

INSOLUBLE DRUG DELIVERY

This invention provides a novel process for producing sub-micron sized particles of water insoluble compounds with biological uses, particularly water insoluble drugs.

BACKGROUND AND SUMMARY OF THE INVENTION

Approximately one-third of the drugs in the United States Pharmacopoeia are water-insoluble or poorly water-soluble. Many currently available injectable formulations of such drugs carry important adverse warnings on their labels that originate from detergents and other agents used for their solubilization. Oral formulations of water-insoluble drugs or compounds with biological uses frequently show poor and erratic bioavailability. In addition, water-solubility problems delay or completely block the development of many new drugs and other biologically useful compounds.

Two alternative approaches for insoluble drug delivery are microparticles which involves forming a phospholipid stabilized aqueous suspension of submicron sized particles of the drug (see U.S. 5,091,187; 5,091,188 and 5,246,707) and microdroplets which involves forming a phospholipid stabilized oil in water emulsion by dissolving the drug in a suitable bio-compatible hydrophobic carrier (see U.S. 4,622,219 and 4,725,442).

The pharmacokinetic properties of both oral and injectable microparticle formulations are dependent on both the particle size and phospholid surface modifier. However, with certain water insoluble compounds the current employed methods of particle size reduction are problematic. Thus, the overall objective of this invention is to develop a novel process based on the use of compressed fluids, including supercritical fluid technology, that yields surface modifier stabilized suspensions of water insoluble drugs with an average particle size of 100 nm to about 300 nm and a narrow size

distribution. The inventive process is robust, scalable and applicable to a wide range of water-insoluble compounds with biological uses.

<div align="center">BRIEF DESCRIPTION OF THE DRAWINGS</div>

The invention is further explained with reference to the attached drawings in which

Figure 1 is a schematic representation of an apparatus for carrying out the present invention by precipitating the bioactive substance by rapid expansion from a supercritical solution;

Figure 2A is a more detailed representation of the preheater assembly of Figure 1;

Figure 2B is an enlarged perspective view of the expansion nozzle of Figure 1;

Figure 3 is a schematic representation of an apparatus for preparing sub-micronsized particles according to the invention by precipitating a bioactive substance, suitably solubilized, into a compressed gas, liquid or supercritical fluid;

Figure 4 is a graph showing the particle size distribution on a volume weighted basis of the cyclosporine particles produced in Example 1 expanded into a phospholipid containing 1 wt% stabilizer;

Figure 5 is a graph showing the particle size distribution on a volume weighted basis of the cyclosporine particles produced in Example 1 expanded into a phospholipid containing 2 wt% stabilizer;

Figure 6 is a graph showing the particle size distribution on a volume weighted basis of the indomethacin particles produced in Example 3 sprayed directly into carbon dioxide;

Figure 7 is a graph showing the particle size Gaussian distribution on a volume weighted basis of the indomethacin particles produced in Example 3 sprayed into a phospholipid containing 2 wt% stabilizer;

<div align="center">2</div>

Figure 8 is a graph showing the particle size distribution on a volume weighted basis of the tetracaine hydrochloride particles produced in Example 4 sprayed into carbon dioxide and water;

Figure 9 is a graph showing the particle size distribution on a volume weighted basis of the tetracaine hydrochloride particles produced in Example 4 sprayed into carbon dioxide and water also containing 1 wt% of stabilizer; and

Figure 10 is a graph showing the particle size Gaussian distribution on a volume weighted basis of tetracaine hydrochloride particles produced in Example 4 sprayed into carbon dioxide, water and 2 wt% stabilizer.

DESCRIPTION OF THE INVENTION

This invention is a process using compressed fluids to produce submicron sized particles of industrially useful poorly soluble or insoluble compounds with biological uses by: (1) precipitating a compound by rapid expansion from a supercritical solution (Rapid expansion from supercritical solution) in which the compound is dissolved, or (2) precipitating a compound by spraying a solution, in which the compound is soluble, into compressed gas, liquid or supercritical fluid which is miscible with the solution but is antisolvent for the compound. In this manner precipitation with a compressed fluid antisolvent (Compressed fluid antisolvent) is achieved. Optionally, the process combines or integrates a phospholipid in water or other suitable surface modifiers such as surfactants, as may be required, into the processes. The surfactant is chosen to be active at the compound-water interface, but is not chosen to be active at the carbon dioxide-organic solvent or carbon dioxide- compound interface when carbon dioxide is used as the supercritical solution. A unique feature of this invention is the combination of either rapid expansion from supercritical solution or compressed fluid antisolvent with recovery of surface modified stable submicron particles in an aqueous phas:-

3

By industrially useful insoluble or poorly soluble compounds we include biologically useful compounds, imaging agents, pharmaceutically useful compounds and in particular drugs for human and veterinary medicine. Water insoluble compounds are those having a poor solubility in water, that is less than 5 mg/ml at a physiological pH of 6.5 to 7.4, although the water solubility may be less than 1 mg/ml and even less than 0.1 mg/ml.

Examples of some preferred water-insoluble drugs include immunosuppressive and immunoactive agents, antiviral and antifungal agents, antineoplastic agents, analgesic and anti-inflammatory agents, antibiotics, anti-epileptics, anesthetics, hypnotics, sedatives, antipsychotic agents, neuroleptic agents, antidepressants, anxiolytics, anticonvulsant agents, antagonists, neuron blocking agents, anticholinergic and cholinomimetic agents, antimuscarinic and muscarinic agents, antiadrenergic and antarrhythmics, antihypertensive agents, antineoplastic agents, hormones, and nutrients. A detailed description of these and other suitable drugs may be found in *Remington's Pharmaceutical Sciences*, 18th edition, 1990, Mack Publishing Co. Philadelphia, PA.

Cyclosporine, a water insoluble immunosuppressive drug, is used as a model to illustrate the invention. This drug was chosen since it has not been possible by using conventional size reduction techniques to achieve the particle size and distribution believed necessary to reach the desired pharmacokinetic performance.

Cyclosporine is a water insoluble, lipophilic 11 amino acid polypeptide with unique immunosuppressive properties. Its major use is as an immunosuppressant in solid organ transplantation. The clinical utility of the currently available pharmaceutical dosage forms are severely limited by the drug's insolubility. That is, the bioavailability of the oral form is low and the intra and inter patient absorption is variable.

The phospholipid may be any natural or synthetic phospholipid, for example phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, phosphatidylinositol, phosphatidylglycerol, phosphatidic acid, lysophospholipids, egg or soybean phospholipid or a combination thereof. The phospholipid may be salted or desalted, hydrogenated or partially hydrogenated or natural semisynthetic or synthetic.

Examples of some suitable second surface modifiers include: (a) natural surfactants such as casein, gelatin, tragacanth, waxes, enteric resins, paraffin, acacia, gelatin, cholesterol esters and triglycerides, (b) nonionic surfactants such as polyoxyethylene fatty alcohol ethers, sorbitan fatty acid esters, polyoxyethylene fatty acid esters, sorbitan esters, glycerol monostearate, polyethylene glycols, cetyl alcohol, cetostearyl alcohol, stearyl alcohol, poloxamers, polaxamines, methylcellulose, hydroxycellulose, hydroxy propylcellulose, hydroxy propylmethylcellulose, noncrystalline cellulose, polyvinyl alcohol, polyvinylpyrrolidone, and synthetic phospholipids, (c) anionic surfactants such as potassium laurate, triethanolamine stearate, sodium lauryl sulfate, alkyl polyoxyethylene sulfates, sodium alginate, dioctyl sodium sulfosuccinate, negatively charged phospholipids (phosphatidyl glycerol, phosphatidyl inosite, phosphatidylserine, phosphatidic acid and their salts), and negatively charged glyceryl esters, sodium carboxymethylcellulose, and calcium carboxymethylcellulose, (d) cationic surfactants such as quaternary ammonium compounds, benzalkonium chloride, cetyltrimethylammonium bromide, chitosans and lauryldimethylbenzylammonium chloride, (e) colloidal clays such as bentonite and veegum. A detailed description of these surfactants may be found in *Remington's Pharmaceutical Sciences*, and Theory and Practice of Industrial Pharmacy, Lachman et al, 1986.

More specifically, examples of suitable second surface modifiers include one or combination of the following: polaxomers, such as Pluronic™ F68, F108

and F127, which are block copolymers of ethylene oxide and propylene oxide available from BASF, and poloxamines, such as Tetronic™ 908 (T908), which is a tetrafunctional block copolymer derived from sequential addition of ethylene oxide and propylene oxide to ethylene-diamine available from BASF, Triton™ X-200, which is an alkyl aryl polyether sulfonate, available from Rohm and Haas. Tween 20, 40, 60 and 80, which are polyoxyethylene sorbitan fatty acid esters, available from ICI Speciality Chemicals, Carbowax™ 3550 and 934, which are polyethylene glycols available from Union Carbide, hydroxy propylmethylcellulose, dimyristoyl phosphatidylglycerol sodium salt, sodium dodecylsulfate, sodium deoxycholate, and cetyltrimethylammonium bromide.

Particles produced by the process of this invention are generally at most 500 nm in size usually below 300 nm, desirably less than 200 nm, preferably less than about 100 nm and often in a range of 0.1 to 100 nm in size. These particles are narrowly distributed in that 99% of the particles are below 500 nm and preferably below 400 nm with peaks at half width at half height at about 200 nm and preferably below 100 nm. The particles may be recovered from suspension by any convenient means such as spray drying, lyophilization, diafiltration, dialysis or evaporation.

The solvent properties of supercritical fluids are strongly affected by their fluid density in the vicinity of the fluid's critical point. In rapid expansion from supercritical solutions, a non volatile solute is dissolved in a supercritical fluid. Nucleation and crystallization are triggered by reducing the solution density through rapid expansion of the supercritical fluid to atmospheric conditions. To achieve this the supercritical fluid is typically sprayed through 10-50 microns (internal diameter) nozzles with aspect ratios (L/D) of 5-100. The fluid approaches sonic terminal velocity at the nozzle tip and high levels of supersaturation result in rapid nucleation rates and limited crystal growth. The combination of a rapidly propagating mechanical perturbation and high

6

supersaturation is a distinguishing feature of rapid expansion from a supercritical solution. These conditions lead to the formation of very small particles with a narrow particle distribution.

The first comprehensive study of rapid expansion from a supercritical solution was reported by Krukonis (1984) [V.J.Krukonis: *AIChE Annual Meeting San Francisco* (1984), as cited in J.W.Tom *et al.: Supercritical Fluid Engineering Science*, Chapter 19, p238, (1993)] who formed micro-particles of an array of organic, inorganic, and biological materials. Most particle sizes reported for organic materials, such as lovastatin, polyhydroxyacids, and mevinolin, were in the 5-100 micron range. Nanoparticles of beta-carotene (300 nm) were formed by expansion of ethane into a viscous gelatin solution in order to inhibit post expansion particle aggregation.

Most rapid expansion from supercritical solution studies on organic materials utilize supercritical carbon dioxide. However, ethane was preferred to carbon dioxide for beta-carotene because of certain chemical interactions. Carbon dioxide is generally preferred, alone or in combination with a cosolvent. Minute additions of a cosolvent can increase the solubility of some solutes by orders of magnitude. When cosolvents are used in rapid expansion from a supercritical solution, care is required to prevent desolution of the particles due to solvent condensing in the nozzle. Normally, this is achieved by heating the supercritical fluid, prior to expansion, to a point where no condensate (mist) is visible at the nozzle tip.

A similar problem occurs when carbon dioxide is used alone. During adiabatic expansion (cooling), carbon dioxide will be in two phases unless sufficient heat is provided at the nozzle to maintain a gaseous state. Most investigators recognize this phenomenon and increase the pre-expansion temperature to prevent condensation and freezing in the nozzle. A significant heat input is required (40-50 kcal/kg) to maintain carbon dioxide in the gaseous

7

state. If this energy is supplied by increasing the pre-expansion temperature the density drops and consequently reduces the supercritical fluid's solvating power. This can lead to premature precipitation and clogging of the nozzle.

There are a number of advantages in utilizing compressed carbon dioxide in the liquid and supercritical fluid states, as a solvent or anti-solvent for the formation of materials with submicron particle features. Diffusion coefficients of organic solvents in supercritical fluid carbon dioxide are typically 1-2 orders of magnitude higher than in conventional liquid solvents. Furthermore, carbon dioxide is a small linear molecule that diffuses more rapidly in liquids than do other antisolvents. In the antisolvent precipitation process, the accelerated mass transfer in both directions can facilitate very rapid phase separation and hence the production of materials with sub-micron features. It is easy to recycle the supercritical fluid solvent at the end of the process by simply reducing pressure. Since supercritical fluids do not have a surface tension, they can be removed without collapse of structure due to capillary forces. Drying of the product is unusually rapid. No carbon dioxide residue is left in the product, and carbon dioxide has a number of other desirable characteristics, for example it is non-toxic, nonflammable, and inexpensive. Furthermore, solvent waste is greatly reduced since a typical ratio of antisolvent to solvent is 30:1.

As an antisolvent, carbon dioxide has broad applicability in that it lowers the cohesive energy of nearly all organic solvents. In 1992, D.J. Dixon, PhD. Dissertation, University of Texas at Austin, described a process in which liquid solutions of polymer in solvent are sprayed into compressed carbon dioxide to form microspheres and fibers. In this process, so called precipitation with a compressed fluid antisolvent, the polymer is insoluble in carbon dioxide, and the organic solvent is fully miscible with CO_2. This concept has been used to form biologically active insulin particles (4 microns) [Yeo, S. D., Lim, G.B. and Debenedetti, P.G. Formation of Microparticulate Protein Powders using a

Supercritical Fluid Anti-Solvent Biotechnol. and Bioeng. 1993, 341], several

micron biodegradable L-poly(lactic acid) particles [Randolph, T. W. B., R.A.;

Johnston, K.P. Micron Sized Biodegradeable Particles of Poly(L-lactic Acid) via

the Gas Antisolvent Spray Precipitation Process. Biotechnology Progress. 1993,

9, 429] and methylprednisolone acetate particles (<5 microns) [W.J. Schmitt, M.

C. S., G.G. Shook, S. M. Speaker. Finely-Divided Powders by Carrier Solution

Injection into a Near or Supercritical Fluid. Am. Inst. Chem. Eng. J. 1995, 41,

2476-2486]. Somewhat surprisingly, the particle sizes have been as small as

those made by rapid expansion from a supercritical solution, despite the

potentially faster times for depressurization in rapid expansion from a

supercritical solution versus two-way mass transfer in the Compressed fluid

antisolvent process. Not only can the compressed fluid antisolvent process

produce PS particles, but also solid and hollow fibers highly oriented microfibrils

biocontinuous networks and 100 nm microballoons with porous shells.

To date, it has not been possible to make submicron particles by the

compressed fluid antisolvent process without particle aggregation or flocculation.

Our objective is to overcome this limitation with the use of surface modifiers, also

termed surfactant stabilizers, such as phospholipids, salts of cholic and

deoxycholic acids, Tweens (polyoxyethylene sorbitan esters), Pluronic F-68,

Tetronic-908, hydroxypropylmethyl cellulose (HPMC), Triton X-100,

cetyltrimethylammonium bromide, PEG-400 or combinations of these compounds

as described in more detail above.

Considerable variations as to the identities and types of phospholipid and

especially the surface active agent or agents should be expected depending upon

the water-insoluble or poorly water-soluble biologically active substance selected

as the surface properties of these small particles are different. The most

advantageous surface active agent for the insoluble compound will be apparent

following empirical tests to identify the surfactant or surfactant

system/combination resulting in the requisite particle size and particle size stability on storage over time.

Appropriate choice of stabilizers will prevent flocculation in the aqueous phase. The surfactant is chosen to be active at the compound water interface, but it is not chosen to be active at the carbon dioxide-organic solvent or carbon dioxide-drug interface. It is not necessary for the stabilizer to be soluble in CO_2; it can be soluble in the liquid to be sprayed, as it only needs to be active at the CO_2/solute interface.

This invention provides a supercritical fluid/compressed fluid based process to produce suspensions of water insoluble drugs with an average particle size of less than 100 nm and a narrow size distribution. An essential element is the use of phospholipids and other surfactants to modify the surface of the drug particles to prevent particle aggregation and thereby improve both their storage stability and pharmacokinetic properties.

DETAILED DESCRIPTION OF THE INVENTION

Materials and methods: Particle sizing was based on the principle of photon correlation spectroscopy using Submicron Particle Sizer-Autodilute Model 370 (NICOMP Particle Sizing Systems, Santa Barbara, CA). This instrument provides number weighted, intensity weighted, and volume weighted particle size distributions as well as multimodality of the particle size distribution, if present.

Separation and quantitation of cyclosporine was carried out with a Waters HPLC system utilizing reverse phase chromatography. The drug was extracted from the sample with methanol and injected for analysis on a C-18 analytical column at 60-80°C with a mobile phase consisting of acetonitrile, methanol, and water. Anylate was detected though its absorbance at 214nm. Operation of the chromatography system and data processing was conducted by Waters Millennium v2.1 software.

Carbon dioxide was used to prepare rapid expansion supercritical solutions since there is no literature reference to any chemical interaction with cyclosporine. Carbon dioxide has been used as a solvent for cyclosporine in fermentation recovery and in HPLC. The relative solubilities of cylclosporine dissolved in a solvent that is expanded with compressed carbon dioxide will be established.

A gas will approach sonic terminal velocity when expanded in a nozzle. Therefore it is important to determine the maximum nozzle diameter and aspect ratio (L/D) that will maintain these conditions in scaleup. Nozzle diameters of 10-50 microns are reported to be used in conjunction with aspect ratios ranging from 5 to 200.

The apparatus for rapid expansion from supercritical solution shown in Figure 1 included a high pressure vessel 1 for formulating the drug/CO_2 solution. Because the drug solution was isolated from the pressurizing fluid by the piston 2 and the valve 2a, the concentration of the drug was constant during the spray. The solution was mixed with a stir bar 14a and a magnetic stirrer 14. The temperature was controlled with heating tape 4. The pressure on the piston and hence the drug solution was controlled via line 3 by an automated syringe pump 5 (ISCO model 100DX) containing pure carbon dioxide.

The preheater as shown in Figure 2A consisted of a hole (0.030" i.d. and 4" long) 8a bored axially along the center of a 2" o.d. x 0.030" i.d. x 4" long copper rod to preheat the solution to a desired temperature before expansion. The preheater assembly 8 and the expansion valve 7 are connected to the high pressure vessel 1 via outlet tube 6. The assembly 8 and the expansion valve 7 were heated with high temperature heating tape 12 and were highly insulated. To monitor the temperature, a thermocouple 13 was placed directly into the preheater assembly close to the orifice.

The expansion nozzle as shown in more detail in Fig. 2B included a 0.254 mm thick, 30 micron diameter laser-drilled orifice 11 (length to diameter ratio ~8.5), which was placed between two copper gaskets 15 (10 mm o.d., 6 mm i.d. and 1 mm thick) and sealed in a 1/4" tubing assembly. The downstream end of the orifice was counterbored into a V-shape as shown in Fig. 2B to prevent the expanding jet from hitting the walls and distorting the morphology of the precipitating solute. To prevent plugging of the orifice, a 1/4" inch diameter, 0.5 micron metal filter 9 was inserted upstream of the nozzle preheater assembly (Figure 1). In addition, a bypass line 10 was used to pre-pressurize the preheater assembly with pure solvent (CO_2) before each spray, otherwise the initial pressure drop across the filter would precipitate the drug and plug the orifice 11. After displacing pure solvent from the preheater, the orifice was submerged into 25 mL aqueous solution in order to trap and stabilize the precipitating drug microparticles. The high kinetic energy of the jet forced the spray 2 cm below the surface of the aqueous phase.

The apparatus used to carry out the Compressed fluid antisolvent sprays is shown in Figure 3. A 300 mL high pressure vessel 16 equipped with a magnetically coupled agitator (Parr) depicted in outline above vessel 16 was used to precipitate the drug. Prior to spraying the drug solution, 50 mL of aqueous solution was added to this precipitator. The aqueous solutions were either pure water, 1.0 wt % Tween 80 in water 10 wt % phospholipid dispersion in water or 10 wt % phospholipid dispersion with 2.0 wt % Tween 80 in water Phospholipid and phospholipid plus Tween-80 dispersions were made by high shear homogenization of their aqueous suspension by passing through a microfluidizer (model M110EH, Microfluidics). Tween-80 was purchased from ICI and egg phospholipid was from Pfansthiel. Aqueous sodium hydroxide solution (1N) was used to adjust the pH of these dispersions to 7.5. Carbon dioxide was compressed with a Haskel air driven gas booster 17 (model AC-152), regulated with a Tescom

12

pressure regulator (model 26-1021) 18 and monitored by pressure gauge 19. The CO_2 pressure was monitored to within ±0.2 bar. A water bath with a recirculator 30 was used to control the precipitator temperature. The solution was sprayed through 50 micron i.d. fused silica capillary tubing 27 (Polymicro Technology) with a length/diameter ratio of 2800. To maintain a constant flow rate, the solution was pumped through the solution valve 28 to the capillary atomizer using an automated syringe pump 20 (ISCO model 100DX).

A 0.5 μm filter 21 was threaded into the CO_2 effluent line 22 to prevent loss of the water insoluble compound from the precipitation vessel. The filter assembly included an in-line sintered filter element (Swagelok "F" series) which was welded onto a 1/4" i.d. NPT fitting. The effluent vent valve 23 (Whitey, SS-21RS4) connected to rotameter 24 was heated in a water bath 29 to at least 50°C to prevent the expanding CO_2 from freezing. During precipitation, a known amount of aqueous solution 25 was agitated using a 45° pitched blade impeller 26. After precipitation, agitation was discontinued and the vessel was isolated to depressurize for 30-45 min. The aqueous solution was then recovered for particle size analysis.

Unless otherwise specified, all parts and percentages reported herein are weight per unit volume (w/v), in which the volume in the denominator represents the total volume of the system. Diameters of dimensions are given in millimeters (mm = 10^{-3} meters), micrometers (μm = 10^{-6} meters), nanometers (nm = 10^{-9} meters) or Angstrom units (= 0.1 nm). Volumes are given in liters (L), milliliters (mL = 10^{-3} L) and microliters (μL = 10^{-6} L). Dilutions are by volume. All temperatures are reported in degrees Celsius. The compositions of the invention can comprise, consist essentially of or consist of the materials set forth and the process or method can comprise, consist essentially of or consist of the steps set forth with such materials.

While the invention has been described in connection with what is presently considered to be the most practical and preferred embodiment, it is to be understood that the invention is not to be limited to the disclosed embodiment, but on the contrary, is intended to cover various modifications and equivalent arrangements included within the spirit and scope of the appended claims.

The following examples further explain and illustrate the invention:

Example 1

Cyclosporine Microparticle Formation by the Rapid Expansion from Supercritical Solution Process

A homogeneous solution of cyclosporine in supercritical CO_2 was expanded by rapid expansion from supercritical solution into various aqueous solutions to study microparticle stabilization. The aqueous solutions were pure water 1.0 wt % Tween 80, phospholipid dispersion or 2.0 wt % Tween 80 with phospholipid dispersion. An amount of 0.0480 g of cyclosporine was charged to a variable volume view cell and 20 mL of CO_2 were added to formulate a 0.25 wt % solution. After the solution came to thermal equilibrium (T=35°C) the cyclosporine/CO_2 solution at 3000 psia was sprayed through a 0.30 µm orifice (L/D of 8) into an aqueous solution for 25 seconds. The pre-expansion temperature was 40°C. The volume weighted particle size of the cyclosporine microparticles expanded into pure phospholipid was 153.7 nm (peak 2) as shown in Figure 4. Most of the mass that constitutes the peak 1 of 20-50 nm diameter may originate largely from the phospholipid; however, this population may also possess some particles that contain cyclosporine. The volume weighted mean particle size of the cyclosporine microparticles expanded into phospholipid dispersion with 2.0 wt % Tween 80 was 80.9 nm (peak 2) as shown in Figure 5. In this case again the smaller peak (26.8 nm) may originate largely from the phospholipid and Tween 80 dispersion and a small fraction of cyclosporine containing particulates. A control experiment was performed in which pure

14

carbon dioxide at 3000 psia was sprayed into the phospholipid dispersion. The mean diameter of the particulates in the dispersion was 9 nm. Therefore, the particles greater than 100 nm in Figures 4 and 5 were not originating from purely the phospholipids, but were drug microparticles. Similarly, for the phospholipid dispersion with 2 wt % Tween 80, the mean diameter of the was 28 nm.

Example 2

Water Insoluble Compound Phase Behavior in Compressed CO_2.

In order to assess whether a particular water insoluble compound should be processed by rapid expansion from supercritical solution or compressed fluid antisolvent, the solubility of the candidate drugs in carbon dioxide was measured. Cyclosporine, nifedipine, piroxicam, carbamazepine, indomethacin and tetracaine HI were studied. To prepare solutions with a constant molar composition, measured amounts of drug and CO_2 were charged to the variable volume view cell from Example 1. To increase the solubility, a cosolvent, i.e., acetone or ethanol, was added to the view cell. The temperature and pressure were varied from 25-45°C and 1200 to 4500 psia, respectively. The phase behavior was determined visually by noting when phase separation occurred as the pressure was slowly reduced at 1-2 psia/sec. Table 1 shows a summary of the solubility behavior in CO_2. Cyclosporine was soluble in CO_2 up to 0.5 wt %. Solutions containing 0.01 wt % carbamazepine, tetracaine HI, nifedipine and piroxicam were insoluble in CO_2. With the addition of 2.40 wt % acetone, 0.026 wt % piroxicam was soluble in CO_2 at 25°C for all pressures down to the vapor pressure of CO_2, which is 930 psia. A solution containing 0.028 wt % nifedipine and 2.26 wt % acetone cosolvent was insoluble in CO_2 at 25°C. At 45°C, the nifedipine was solvated with no visible phase separation down to 2000 psia.

SOLUTE	CONC. (wt%)	TEMP. (°C)	CLOUD POINT (psia)
Cyclosporine	0.25	25	soluble down to 1200
Cyclosporine	0.25	30	1850
Cyclosporine	0.25	35	2060
Piroxicam	0.069	25	insoluble up to 4500
Nifedipine	0.088	25	insoluble up to 4000
Nifedipine	0.029 (a)	25	insoluble up to 3500
Carbamazepine	0.0085	25, 40	insoluble up to 4500
Tetracaine Hl	0.0097	25, 45	insoluble up to 4500
Indomethacin	0.0098	25	insoluble up to 4000

(a) with 2.0% ethanol as a co-solvent.

Example 3

Indomethacin Microparticle Formation by the Compressed fluid antisolvent Process

A 9.9 wt % solution of indomethacin in acetone was sprayed into carbon dioxide with the aqueous solution using the Compressed fluid antisolvent process. The duration of the spray was 30 s at 1 mL/min. The volume weighted mean particle size of the phospholipid dispersion was 26 nm (peak 1) as shown in Figure 6. A bimodal size distribution was observed for the indomethacin particles with mean diameters of 143.0 nm (peak 2) and 1088.9 nm (peak 3), respectively. Particles with such a size difference are easily separated by filtration. For the microparticles precipitated into phospholipid dispersion in the presence of 2.0 wt

% Tween 80, the volume weighted mean particle diameter was 126 nm as shown in Figure 7.

Example 4

Tetracaine HI Microparticle Formation by the Compressed fluid antisolvent Process

A 0.97 wt % solution of Tetracaine HI in acetone was sprayed into the precipitator containing carbon dioxide and pure water. The volume weighted mean particle sizes of the Tetracaine HI microparticles were 31.8, 193.4 and 2510.1 nm, respectively (Figure 8). This illustrates that the Compressed fluid antisolvent process can produce extremely small particles even without surfactant stabilizer. With 1.0 wt % Tween 80 added to the water, three peaks were observed with mean diameters of 9.5 nm, 38.3 nm and 169.1 nm (Figure 9). The particle size distribution for 1.0 wt % Tetracaine HI stabilized with phospholipid dispersion and 2.0 wt % Tween 80 is shown in Figure 10. A monomodal distribution is observed between 8-200 nm with a mean diameter of 27.3 nm. This peak includes both the surfactant aggregates and drug particles. No drug particles above 200 nm were observed.

WHAT IS CLAIMED IS:

1. A process of preparing microparticles up to 300 nm in size of water-insoluble or substantially water-insoluble biologically active compounds comprising the steps of :

(1) dissolving a water-insoluble or substantially water-insoluble biologically active compound in a solvent therefor to form a solution; and

(2) spraying the solution prepared in step (1) into a compressed gas, liquid or supercritical fluid in the presence of a surface modifier dispersed or dissolved in an aqueous phase.

2. A process of preparing microparticles up to 300 nm in size of a water-insoluble or substantially water-insoluble biologically active compound comprising the steps of:

(1) dissolving a water-insoluble or substantially water-insoluble biologically active compound in a compressed fluid;

(2) preparing an aqueous phase containing a surface modifier active at the compound-water interface; and

(3) spraying the compressed fluid of step (1) into the aqueous phase of step (2) to form microparticles of the compound.

3. The process according to claim 1 or 2, including the additional step of recovering the microparticles so produced.

4. The process according to claim 1 or 2, wherein the surface modifier is a phospholipid.

5. The process according to claim 1 or 2, wherein the surface modifier is a surfactant.

6. The process according to claim 1 or 2, wherein the surface modifier is a mixture of two or more surfactants.

7. The process according to claim 1 or 2, wherein the surface modifier is at least one surfactant devoid or substantially completely devoid of phospholipids.

8. The process of claim 1 or claim 2 wherein the surface modifier is a polyoxyethylene sorbitan fatty acid ester, a block copolymer of ethylene oxide and propylene oxide, a tetrafunctional block copolymer derived from sequential addition of ethylene oxide and propylene oxide to ethylenediamine, an alkyl aryl polyether sulfonate, polyethylene glycol, hydroxy propylmethylcellulose, sodium dodecylsulfate, sodium deoxycholate, cetyltrimethylammonium bromide or combinations thereof.

9. The process of claim 1 or 2 wherein the surface modifier is of egg or plant phospholipid or semisynthetic or synthetic in partly or fully hydrogenated or in a desalted or salt phospholipid such as phosphatidylcholine, phospholipon 90H or dimyristoyl phosphatidylglyerol sodium salt, phosphatidylethanolamine, phosphatidylserine, phosphatidic acid, lysophospholipids or combinations thereof.

10. The process of claim 1 or 2 wherein the compound is a cyclosporine, indomethacin, or tetracaine.

11. The process of claim 1 or 2 wherein the particles are less than 100 nm in size.

12. The process of claim 1 or 2 wherein the particles range from 5 up to about 50 nm in size.

19

13. The process of claim 1 or 2 wherein 99% of the particles produced are below 500 nm.

14. The process of claim 1 or 2 wherein 99% of the particles produced are below 400 nm with peaks at half width at half height at about 200 nm.

15. The process of claim 14 when the peaks are below 100 nm.

16. The process of claim 1 or 2 wherein the compressed gas or fluid is gas, liquid or supercritical carbon dioxide.

17. The process according to claim 2, wherein the compressed fluid sprayed in step (3) is sprayed through a capillary orifice.
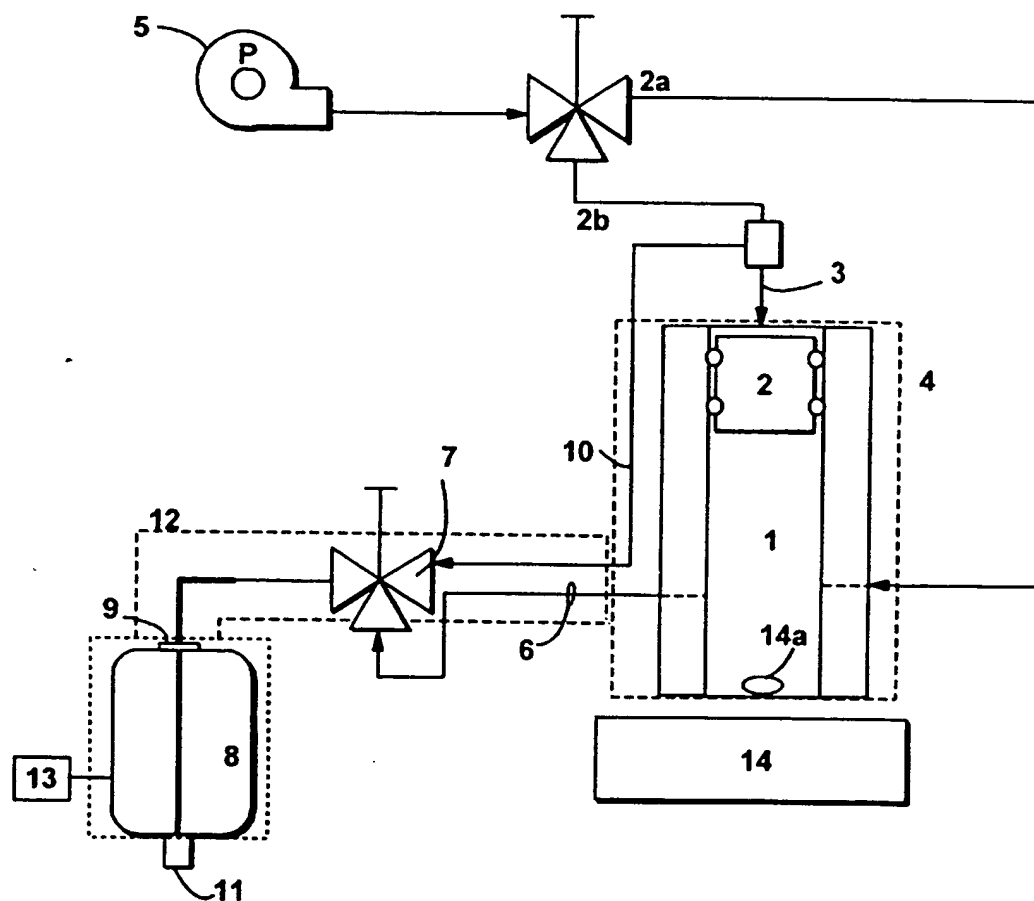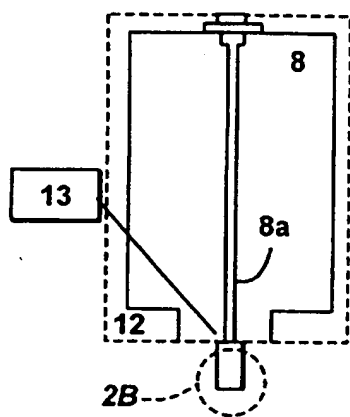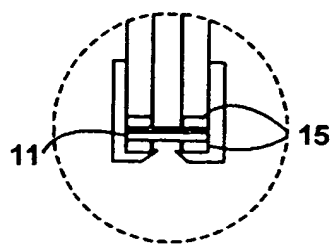
Fig. 1

Fig. 2A

Fig. 2B

Fig. 3
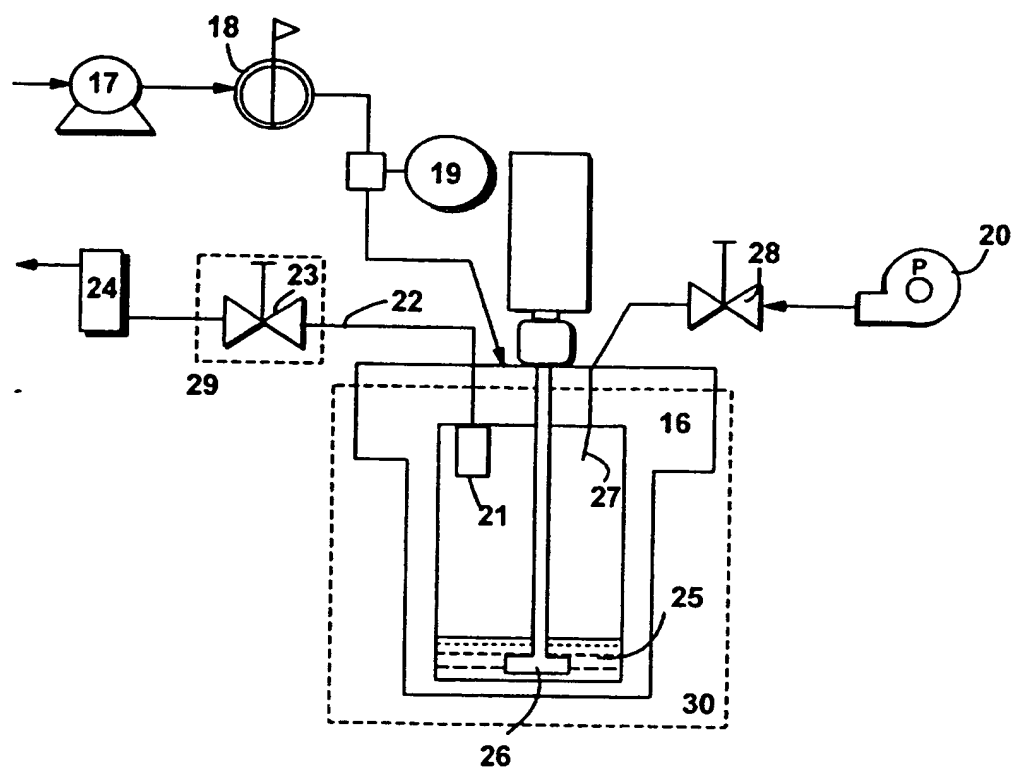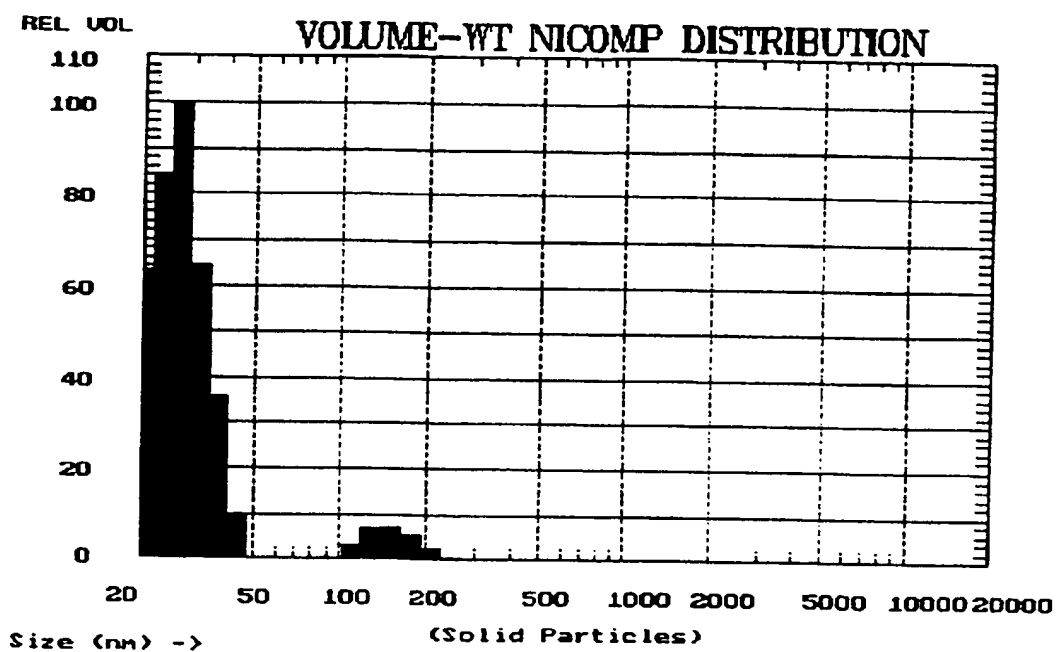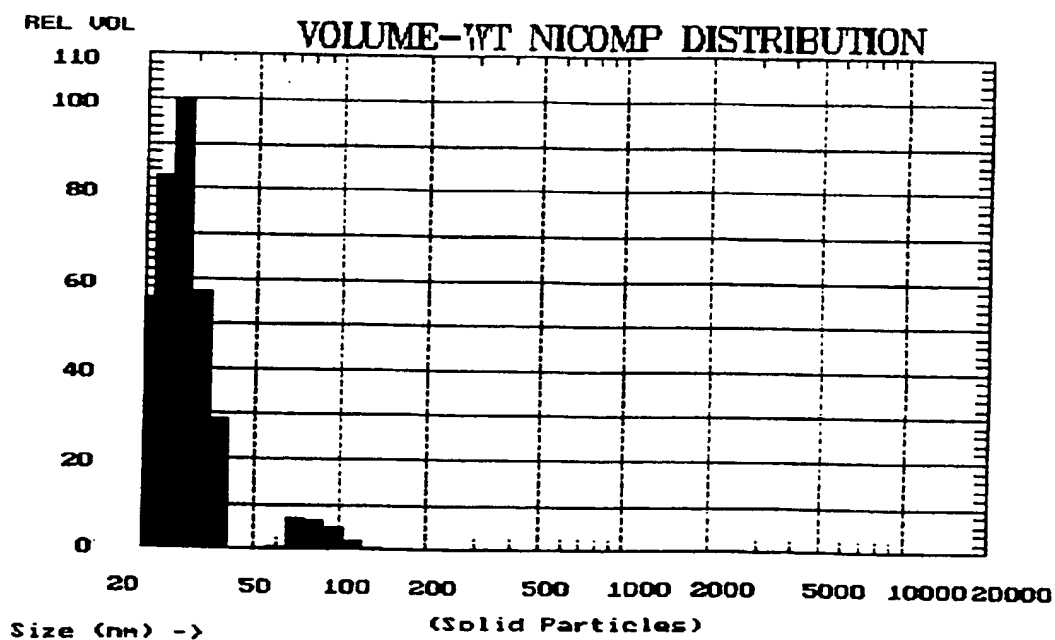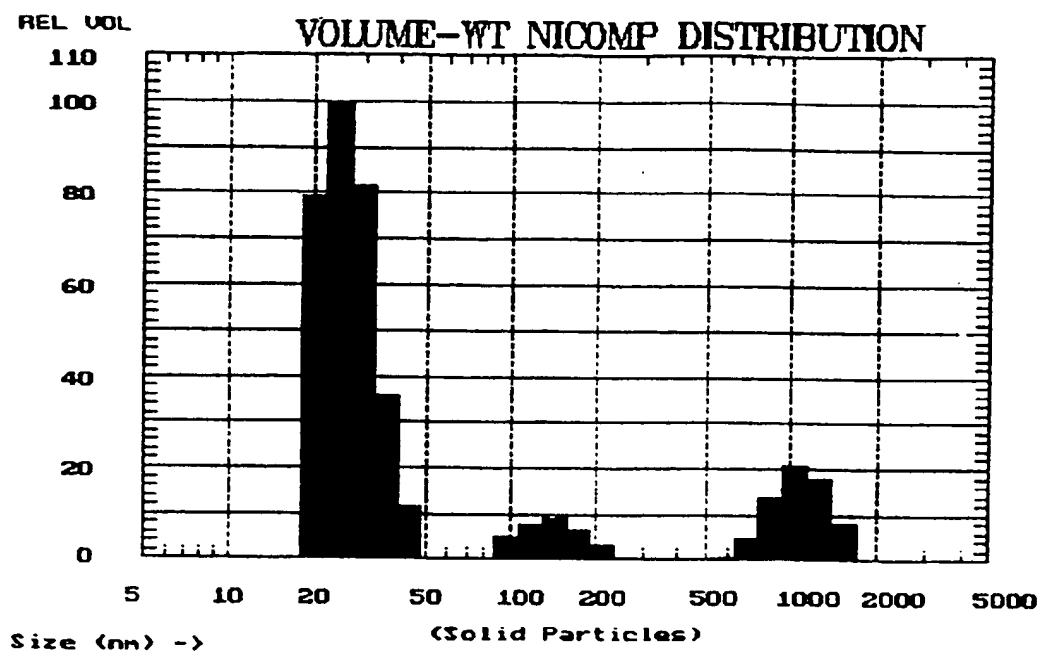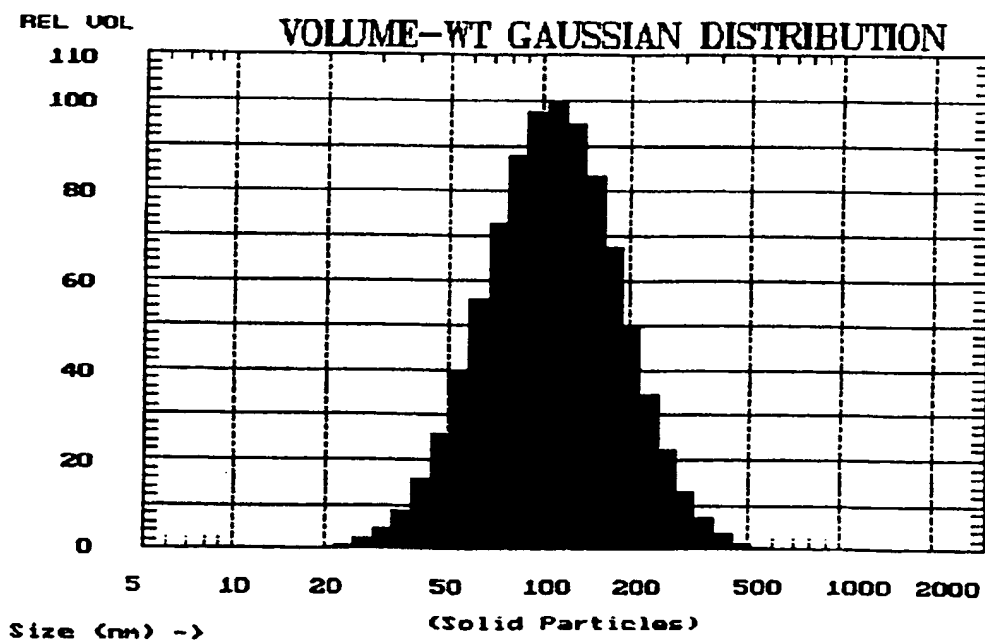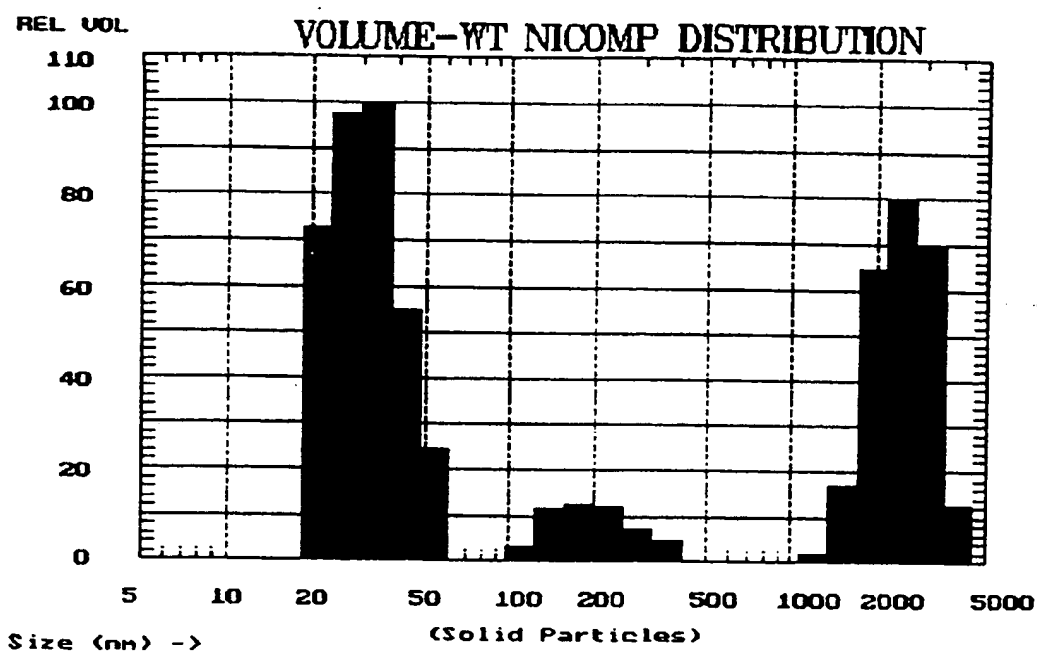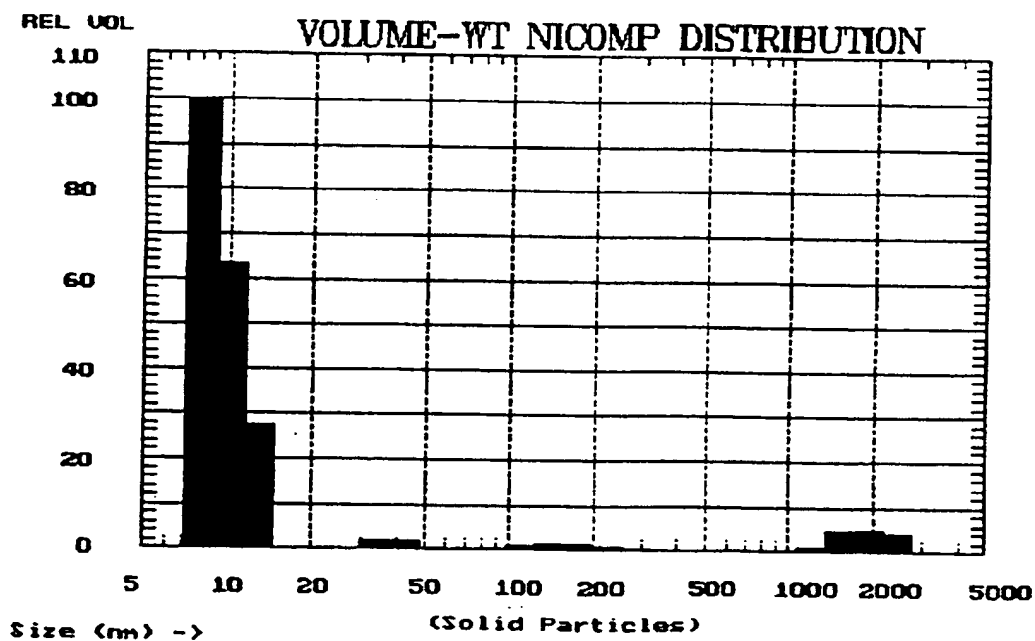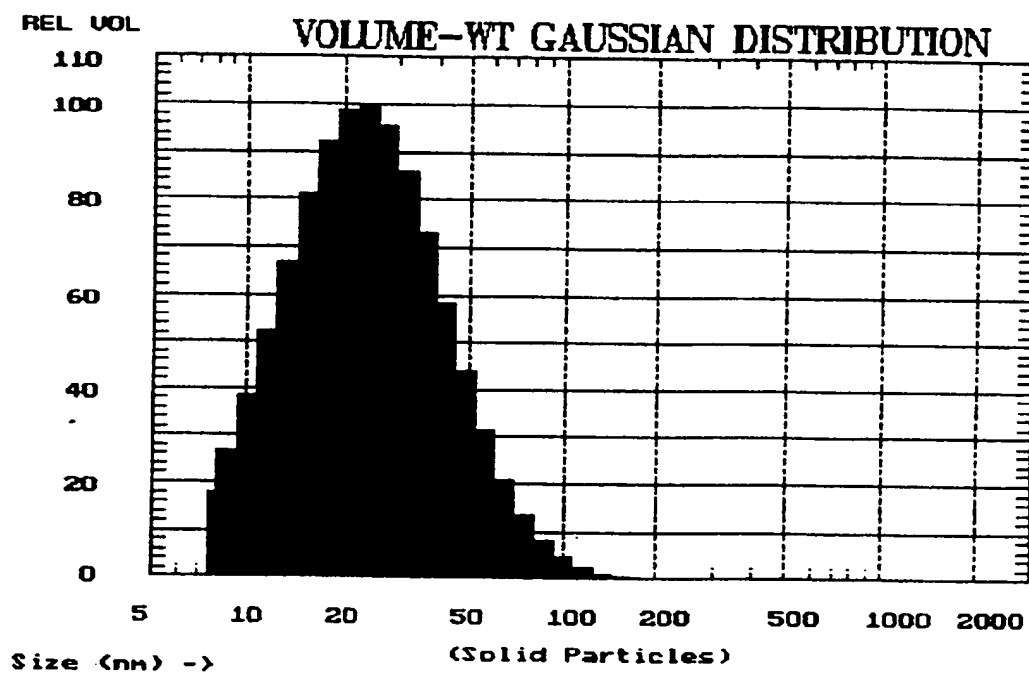
Fig. 4

Fig. 5

Fig. 6

Fig. 7

Fig. 8

Fig. 9

Fig. 10

VOLUME-WT GAUSSIAN DISTRIBUTION

REL VOL

Size (nm) ->   (Solid Particles)

INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6   A61K9/14

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6   A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	EP 0 542 314 A (UNIV PRINCETON) 19 May 1993 see column 1 - column 2; claims 1-12 ---	1-17
Y	EP 0 322 687 A (SANOL ARZNEI SCHWARZ GMBH) 5 July 1989 see claims 1-11 ---	1-17
Y	INTERNATIONAL JOURNAL OF PHARMACEUTICS, vol. 94, 1993, pages 1-10, XP002027507 PHILLIPS E.M. ET AL: "Rapid expansion from supercritical solutions: applications to pharmaceutical processes" see page 7 - page 8 ---	1-17

-/--

| X | Further documents are listed in the continuation of box C. | | X | Patent family members are listed in annex. |

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
18 March 1997	0 4. 04. 97

| Name and mailing address of the ISA
European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (- 31-70) 340-2040, Tx. 31 651 epo nl,
Fax (- 31-70) 340-3016 | Authorized officer

Seegert, K |

Form PCT ISA 210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	EP 0 601 618 A (STERLING WINTHROP INC) 15 June 1994 see page 2, line 6 - line 25; claims 1-18 ---	1-17
P,Y	EP 0 706 821 A (MICROENCAPSULATION CENTRE) 17 April 1996 see column 1, line 5 - line 18 see column 4, line 40 - line 42 -----	1-17

1

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

INTERNATIONAL SEARCH REPORT

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0542314 A	19-05-93	AU 668367 B AU 2831092 A CA 2083027 A JP 5293361 A	02-05-96 20-05-93 15-05-93 09-11-93
EP 0322687 A	05-07-89	DE 3744329 A DE 3880808 A ES 2056890 T JP 2004439 A US 5043280 A	06-07-89 09-06-93 16-10-94 09-01-90 27-08-91
EP 0601618 A	15-06-94	US 5336507 A AU 662453 B AU 5046893 A CA 2102267 A CZ 9302602 A FI 935305 A HU 65758 A JP 6211646 A NO 934204 A NZ 250062 A SK 139093 A US 5470583 A	09-08-94 31-08-95 23-06-94 12-06-94 15-06-94 12-06-94 28-07-94 02-08-94 13-06-94 27-04-95 07-12-94 28-11-95
EP 0706821 A	17-04-96	WO 9611055 A	18-04-96